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13. ABSTRACT *(Maximum 200 Words)*

The primary focus of this project is the first step in the aberrant cell signaling pathways that lead to uncontrolled proliferation and cancer, namely the interaction of growth factors with their receptor tyrosine kinases (RTKs). Our principal goal is to design growth factor antagonists that can inhibit ligand-induced receptor activation as a route to novel anti-cancer drugs. During the past year we have made important progress is establishing the goals of this project. We have developed efficient and direct synthetic routes to protein binding agents. We have extended our approach with the synthesis of some tetraphenylporphyrin based receptors that bind to proteins with high affinity. Most importantly, we have demonstrated that a member of our first generation of protein binding agents is able to bind to the surface of platelet derived growth factor. This binding is strong (IC50 < 250nM) under physiological conditions and is sufficient to block the activation of PDGF receptor tyrosine kinase. These results represent important steps is establishing the novel approach to breast cancer treatment that is the foundation of this project.

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Andrew D Hawslre 5/23/00

3

Table of Contents

5) Introduction

The primary focus of this project is the first step in the aberrant cell signaling pathways that lead to uncontrolled proliferation and cancer, namely the interaction of growth factors with their receptor tyrosine kinases (RTKs). Overexpression of RTKs as well as high serum levels of of the activating growth factor are seen in certain breast and ovarian carcinomas. Furthermore, elevated levels of RTKs in breast cancer patients correlate with poor response to chemotherapy and shorter survival times. The design of growth factor antagonists that can inhibit ligand-induced receptor activation is a potentially novel route to new anti-cancer drugs. In recent years this strategy has been supported by the development of antibodies (e.g. Herceptin) against RTKs that have been shown to be active against breast cancer in the clinic. The main goals of this project are to design, synthesize and evaluate a novel series of synthetic agents that bind to the surface of growth factors and block their interaction with their RTKs. We have recently prepared the first of a new class of protein surface receptors involving the attachment of four peptide loops to a central scaffold (based on the calix[4]arene unit).[1] A key component of the first year of this project was to determine the effectiveness of this strategy and to identify alternative designs that might be similarly successful in the recognition of protein surfaces.

6) Body

Task 1. To further refine the first generation of protein surface recognition agents based on four β-turn cyclic peptide mimetics linked to a calix[4]arene core scaffold (months 1-12).

We have made excellent progress in the completion of task 1. We have prepared a large number of cyclic peptide derivatives based on the 3-aminomethylbenzoic acid scaffold and attached them to the core calixarene scaffold. A selection of these sequences is shown in Figure 1.

Figure 1

However, the calixarene unit suffers several complications, including difficulty of synthesis and tendency to aggregation. We have improved this synthetic approach by studying different organic scaffolds onto which recognition sites can be attached. Porphyrins, owing to their large size, rigidity and photophysical properties are interesting candidates as the central core scaffold component. During the past year we have investigated the design, synthesis and recognition properties of a series of tetraphenylporphyrins that bind, in certain cases with high affinity, to protein surfaces. We have tested the protein binding of these molecules against cytochrome c. Cytochrome c is a particularly attractive target since it is a well-characterized protein that plays a critical role, by binding to Apaf1, in initiating programmed cell death or apoptosis. The porphyrin-based receptors were prepared by the generation of the tetra-acid chloride ((COCl)$_2$, DMF) starting from m-tetrakis-(4-carboxyphenyl) porphyrin **1**, and subsequent coupling with the corresponding t-butyl protected amino acid or peptide amines. Deprotection of t-butyl groups with trifluoroacetic acid provided receptors **2-4**. Acidic and hydrophobic attachments were chosen to complement the cationic and hydrophobic surface surrounding the RTK binding region of many growth factors, e.g. PDGF.

Compounds **1-4**, and coproporphyrin I, a naturally occuring tetraanionic porphyrin, were initially screened for binding to horse heart ferricytochrome c using fluorescence spectroscopy. Addition of cytochrome c to solutions containing **1-4** resulted in quenching of porphyrin fluorescence emission (ex=420nm, em=650nm) due to the enforced proximity of the Fe(III)

heme that results from complex formation between the protein and the receptors. In contrast, titrations with tetracationic m-tetrakis-(4-trimethylaminophenyl) porphyrin (TTMAPP), showed no quenching even at high concentrations indicating the absence of nonspecific binding. Typical titrations of **3, 4** and TTMAPP with cytochrome c are shown in Figure 2.

Figure 2

Receptor **4** contains eight negatively charged groups and 8 phenyl groups. The titration curve (Figure 2) shows a sharper achievement of saturation with **4** compared to **3**. This corresponds to a K_d of 20 ±5 nM for **4** binding to cytochrome c and represents an eight fold increase in affinity compared to **3**. To our knowledge, **4** is one of the strongest synthetic receptors for cytochrome c, under these experimental conditions. The binding experiments between **4** and cytochrome c were carried out in 5mM phosphate buffer at pH 7.4. Remarkably, receptor **4** with a molecular weight of ~1900 Da can bind to cytochrome c 100 fold stronger than its natural protein partners such as cytochrome c peroxidase, which has a K_d of 2.4μM measured at 5mM phosphate buffer, pH=7.2 Full details of this work are described in a paper in press in *Organic Letters* (see appendix).

Task 2. To develop a combinatorial libraries of protein surface binding agents that can the be targeted to a range of growth factor structures (months 12-24).

This task will be an important focus during the second year of this project.

Task 3. To develop synthetic antagonists for PDGF activation of its receptor tyrosine kinase that are active both in purified protein and in whole cell assays (months 1-18).

This task is of central importance to the success of the project and we have made excellent progress towards its completion during the last year. To design molecules that bind PDGF and disrupt its signaling function, we have prepared a novel series of protein surface binding agents. The molecules are composed of a central calix[4] arene scaffold to which is attached four peptide loop domains (Figure 3). The resulting molecules contain a functionalized and variable surface approximately 500Å^2 in area. Several peptide loop sequences were synthesized (Table 1) to provide molecular surfaces with negatively and positively charged as well as hydrophobic regions designed to bind to complementary areas on the target growth factor.

Figure 3

To identify PDGF binding molecules from this library we first used a simple and cost effective cell-based screening assay with NIH 3T3 cells to detect molecules capable of blocking PDGF-BB-induced tyrosine autophosphorylation of the PDGF receptor. The ability of the identified molecules to bind PDGF-BB and inhibit interaction with its receptor is then confirmed biochemically. Starved NIH 3T3 cells were pretreated for 5 min with the synthetic molecules prior to stimulation with PDGF for 10 min. The effects of the molecules on PDGF-stimulated receptor tyrosine phosphorylation were determined by antiphosphotyrosine Western blotting. Screening the library for potential PDGF binders identified GFB-111 as a potent (IC_{50} = 250nM) inhibitor of PDGF-BB-stimulation of PDGF receptor tyrosine autophosphorylation (Table 1). GFB-111 has four-fold symmetry containing 4 identical peptide loops with negative and

hydrophobic residues, GlyAspGlyTyr (GDGY), that are designed to be complementary to the PDGF surface involved in binding to PDGFR. The area of PDGF-BB that

Table 1: Inhibition of PDGF-dependent receptor tyrosine phosphorylation by growth factor binders (GFBs)

Compounds	Loop Sequence	IC$_{50}$ (μM)
GFB-105	GDFD	2.4
GFB-106	GDDD	8
GFB-107	GDGD	2.5
GFB-108	D-ADGD	9
GFB-109	GDLD	7.5
GFB-110	GDAD	1.7
GFB-111	GDGY	0.25
GFB-112	ADGD	29
GFB-113	GDSD	2.8
GFB-115	GKGF	50
GFB-116	GKGK	40
GFB-117	GDND	5.8
GFB-119	PDGD	20
GFB-120	GDDG	1.3
GFB-122	GDDY	1.7

binds PDGFR is composed primarily of positive and hydrophobic residues.[3] Substitution of aspartic acid in the loop by positively charged lysine is not tolerated (compare GFB-111 (GDGY) (IC$_{50}$ = 250nM) to GFB-115 (GKGF) (IC$_{50}$ = 50μM) and GFB-116 (GKGK) (IC$_{50}$ = 40μM)), indicating that negatively charged residues are important for the inhibitory activity of GFB-111. However, negatively charged residues are not sufficient and the presence of an aromatic hydrophobic residue in the loop is also critical ((compare GFB-111(GDGY) to GFB-107 (GDGD) (IC$_{50}$ = 2.5μM) as well as GFB-122 (GDDY) (IC$_{50}$ = 1.7μM) to GFB-106 (GDDD) (IC$_{50}$ = 8μM)) (Table 1).

The complementarity of the recognition domain of GFB-111 (negatively charged and hydrophobic residues) with the receptor binding surface of PDGF (positively charged and hydrophobic sites) suggested that GFB-111 would block binding of PDGF to PDGFR. To support this, we determined whether GFB-111 can block [125-I]-PDGF-BB specific binding in NIH 3T3 cells. Figure 4 shows that in the absence of GFB-111, NIH3T3 cells bound [125-I]-PDGF-BB. However, pre-treatment with increasing concentrations of GFB-111, resulted in a concentration-dependent decrease in PDGF-BB binding. The IC$_{50}$ value of binding inhibition was 250nM (Figure 4) which is similar to the IC$_{50}$ value measured for GFB-111 inhibition of PDGF-BB-stimulated PDGFR tyrosine phosphorylation (Table 1). Figure 4 also shows that PDGF-BB binding to its receptor was blocked by GFB-111 but not GFB-116. Similar experiments with EGF, IGF-1 and VEGF (vascular endothelial growth factor) demonstrated that GFB-111 (10μM) had no significant effects on [125-I]-EGF or [125-I]-IGF-1 binding but

inhibited [I-125]-VEGF binding by 50% in NIH 3T3 cells overexpressing EGFR, IGF-1R and VEGFR (Flk-1), respectively (Figure 5).

We next determined the selectivity of GFB-111 by evaluating its ability to block growth factor-stimulated tyrosine phosphorylation of several receptor tyrosine kinases and subsequent activation of two mitogen-activated protein kinases (MAPK), Erk1 and Erk2. Figure 6 shows that PDGF-BB-stimulation of starved NIH 3T3 cells in the absence of GFB-111, resulted in PDGFR autophosphorylation and Erk1/Erk2 activation. However, pre-treatment of these cells with GFB-111 (0.1 to 10μM) for 5 min resulted in a concentration-dependent inhibition of PDGF-BB stimulation of receptor tyrosine phosphorylation with an IC_{50} of 250nM and a complete block at 3 μM GFB-111. Figure 6 also shows that GFB-111 is a potent inhibitor of PDGF-dependent activation of Erk1 and Erk2 (IC_{50} of 0.8 and 1.2 μM, respectively). PDGF-AA stimulation of PDGF receptor phosphorylation and Erk1/Erk2 activation was also blocked with GFB-111 with a similar potency. In contrast, GFB-111 at concentrations as high as 100μM did not affect the ability of EGF to stimulate EGFR tyrosine phosphorylation and Erk1/Erk2 activation. For comparison, PD158780, a known EGFR tyrosine kinase inhibitor, blocks EGFR tyrosine phosphorylation and Erk1/Erk2 activation (Figure 6).

Task 4. To target other growth factors involved in aberrant breast cell proliferation pathways, including VEGF, EGF and heregulin (months 18-36).

This task will be a focus in the second half of this project.

7) Key Research Accomplishments

- Developed synthetic routes to a wide range of peptide loop derivatives for attachment to core calixarene scaffolds.

- Designed, synthesized and evaluated a novel series of protein surface receptors based on a tetraphenylporphyrin scaffold.

- Showed that certain tetraphenylporphyrin-based receptors can bind with extremely high affinity (Kd <25nM) to model protein targets.

- Established that one member of our first series of protein binding agents is able to bind to platelet derived growth factor and inhibit it from binding and activating its receptor tyrosine kinase.

8) Reportable Outcomes

Manuscripts:
Jain, R.; Hamilton, A. D. "Protein Surface Recognition by Synthetic Receptors Based on a Tetraphenylporphyrin Scaffold", *Organic Letters*, In press.

9) Conclusions

During the past project period we have made important progress is establishing the basic tenet of this project. We have significantly advanced our synthetic work on the design of agents for the complexation of protein exterior surfaces. We have further extended our design strategy with the synthesis of some tetraphenylporphyrin based receptors that bind to model protein surfaces with exceptionally high affinity. These molecules have much lower molecular weights than our original series and so have the potential to possess improved pharmacokinetic and bioavailability properties. Most importantly, we have demonstrated that one molecule from our first generation of protein binding agents is able to bind to the surface of platelet derived growth factor. This binding is strong (IC50 < 250nM) under physiological conditions and is sufficient to block the activation of PDGF receptor tyrosine kinase. These results represent important steps is establishing the novel approach to breast cancer treatment that is the foundation of this project. We can bind to oncogenically important growth factors and block their function in cell culture assays. We are currently extending these studies to establish the extent of growth factor antagonism in mouse xenograft models of human cancer.

1. R$_{1-4}$ = -COOH

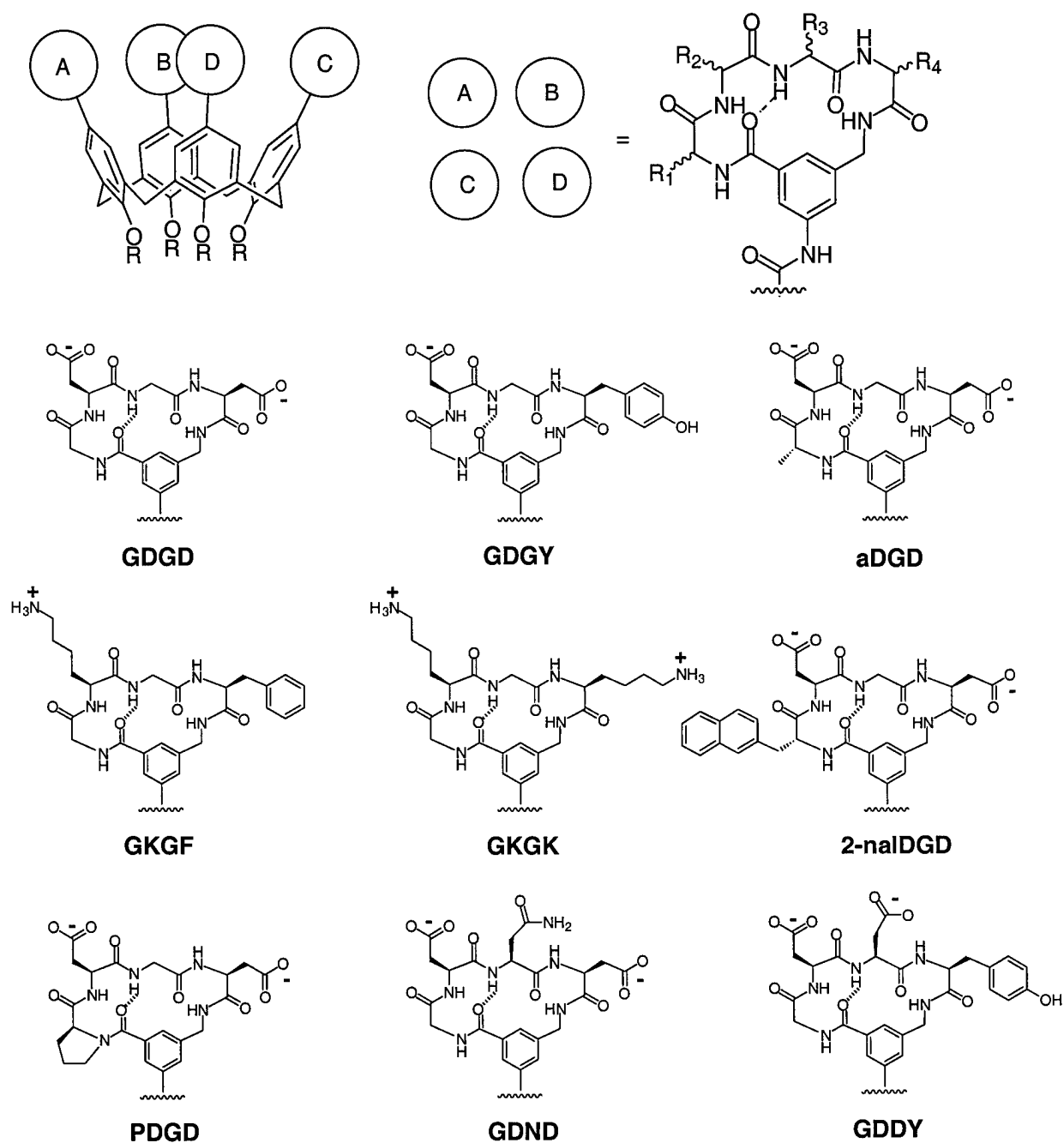**2.** R$_{1-4}$ =

3. R$_{1-4}$ =

4. R$_{1-4}$ =
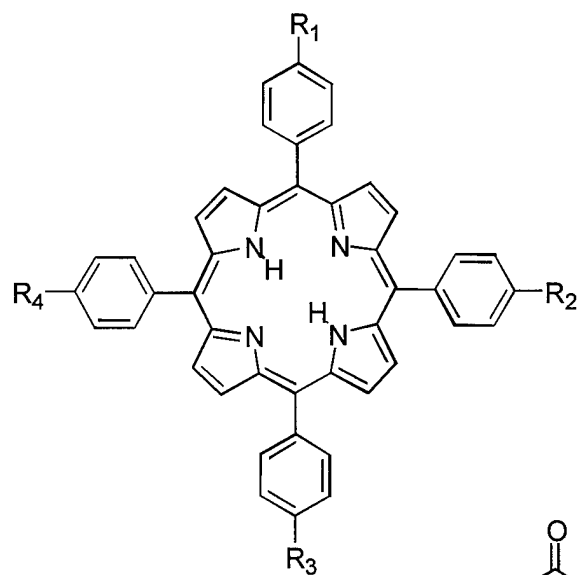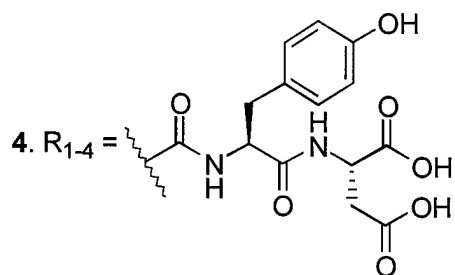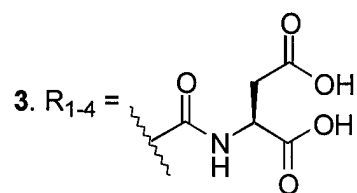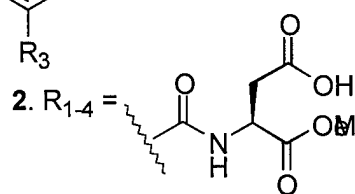
Figure 1. Recent progress in the synthesis of cyclic peptide derivatives and their incorporation in calixarene-based growth factor binding agents.
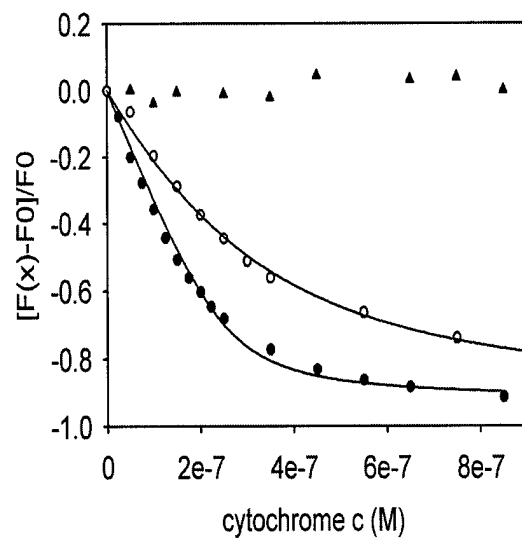
11

Figure 2. Fluorescence quenching of **4** (●), **3** (o) and TTMAPP (▲) upon addition of cytochrome c. The curve fit indicates a K_d of 20 ±5nM for **4** and 160 ±20nM for **3**. Titrations were carried out under 250nM initial receptor concentration in 5mM sodium phosphate buffer, pH = 7.4, at 298K.

Figure 3. Design of protein binding agents for antagonism of PDGF function.

Calix[4]arene

Peptide loop
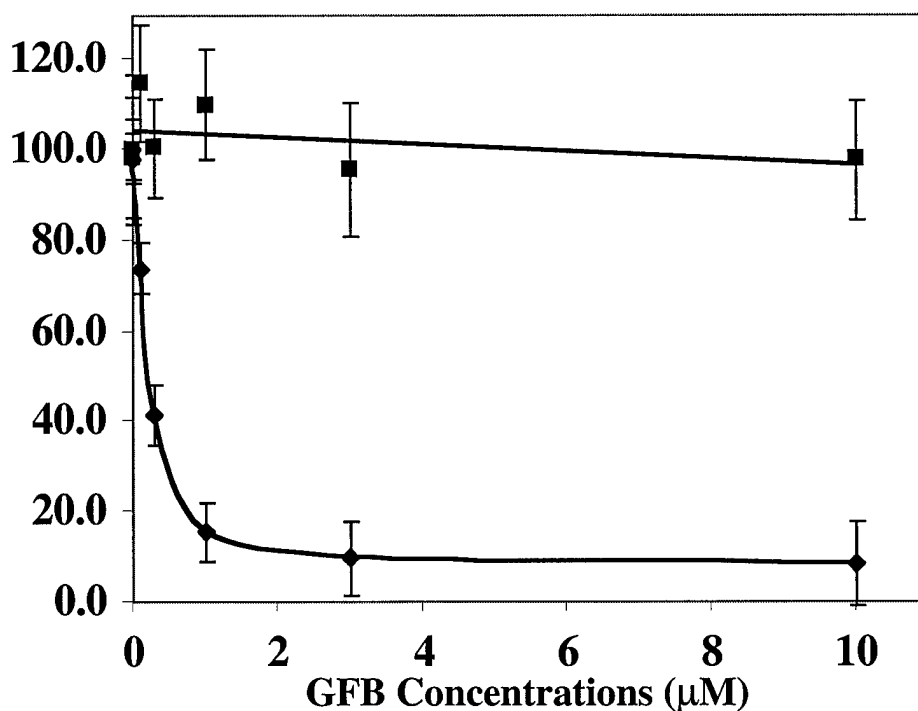
Protein surface binding agent

GDGD

GDGY

GKGF

GKGK
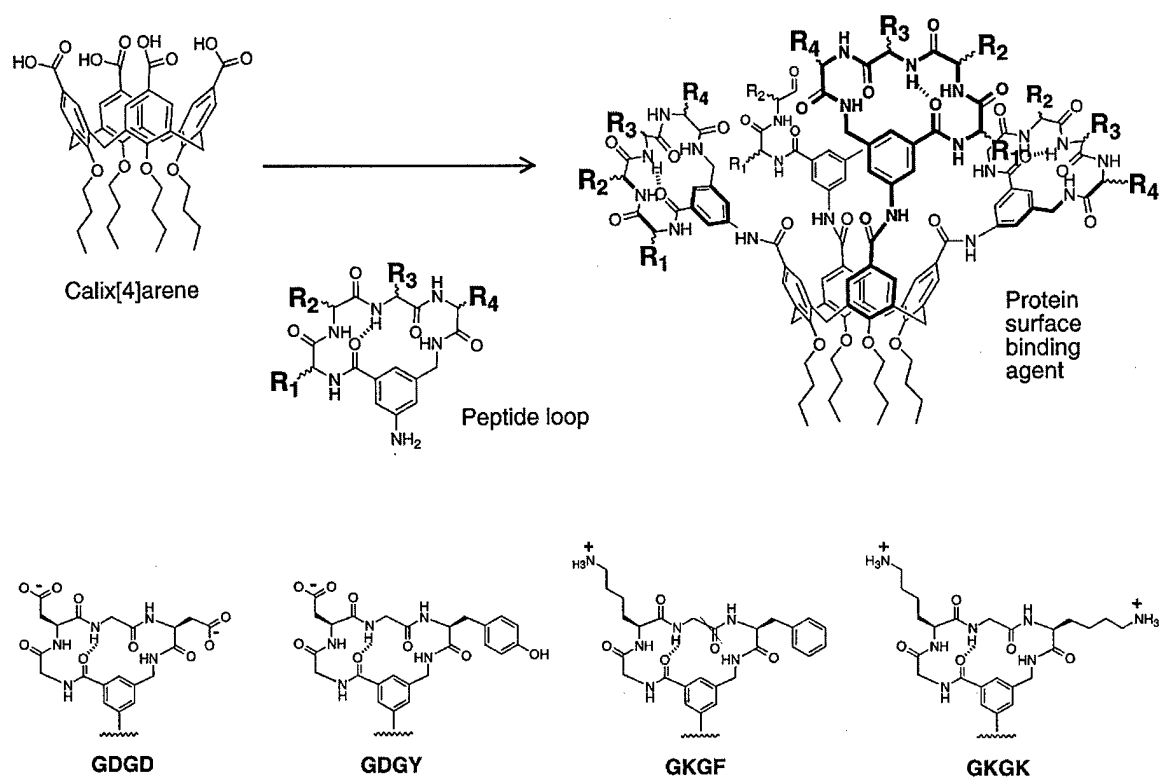
Figure 4: GFB-111 inhibits [125-I]PDGF to its receptor on NIH 3T3 cells. NIH 3T3 (PDGF) or NIH 3T3 cells overexpressing either EGFR (EGF), IGFR (IGF) or Flk-1 (VEGF), were incubated with [125I]-PDGF, [125I]-EGF, [125I]-IGF or [125I]-VEGF, respectively and increasing concentrations of GFB-111 (◆) (C and D) or GFB-116 (■) (C). Cells were incubated at 4°C degrees for 1-3h and washed 3 times with PBS and three times with Tris 25mM pH8.0, 1%Tx-100, 10% Glycerol, 1%SDS. Lysates plus wash were counted. Excess of cold growth factors was used to obtain non-specific binding levels. Data are representative of three independent experiments.

Figure 5. Observed selectivities for GFB-111 binding to different growth factors.
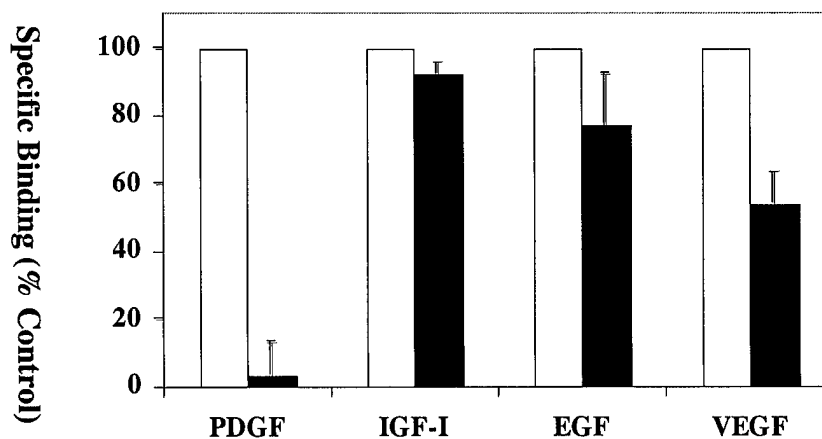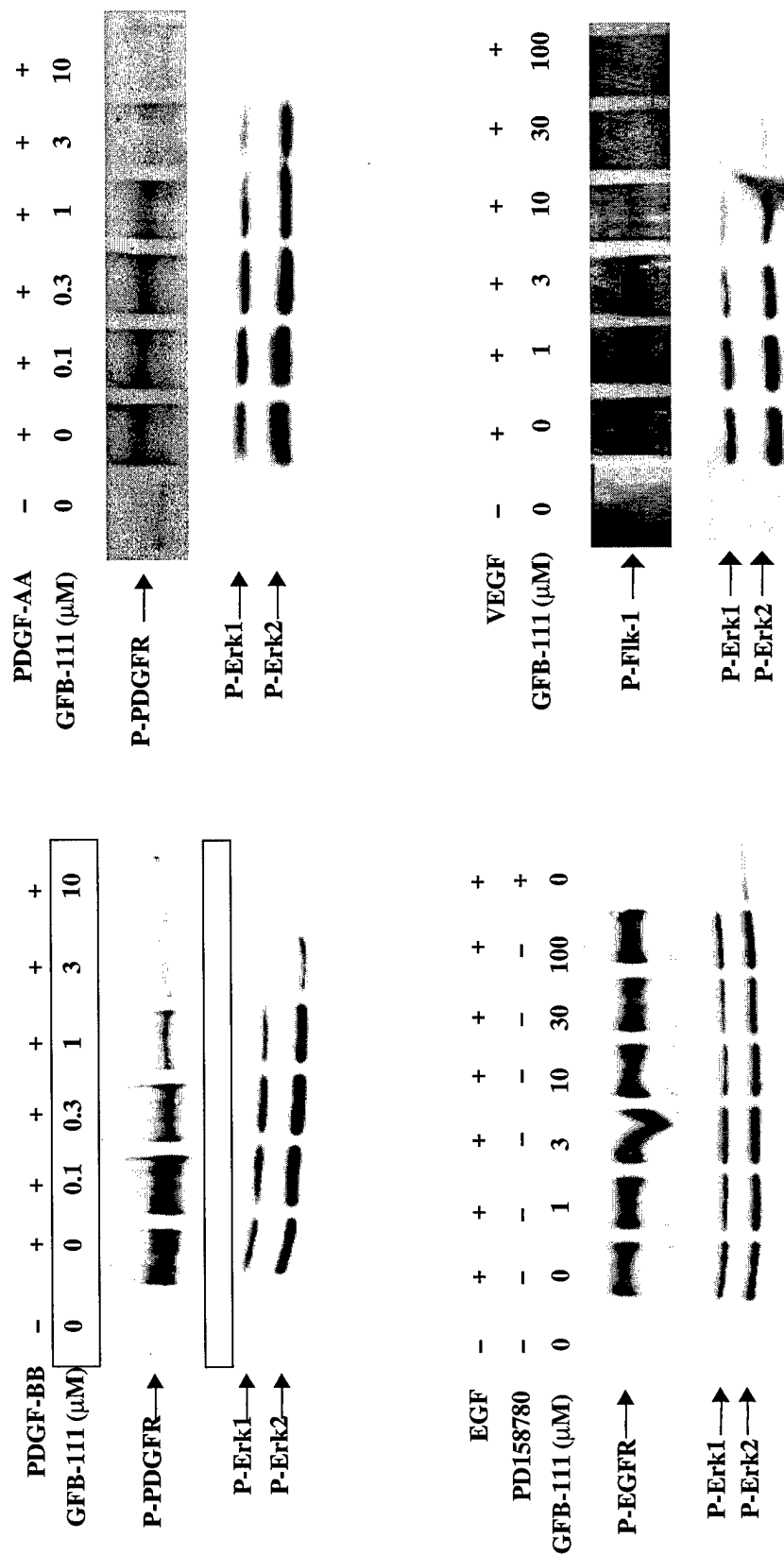
*Figure 6. GFB-111 Inhibits PDGF-BB-, PDGF-AA-, VEGF-,
But Not EGF-Dependent Signaling*

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1. Park, H. S.; Lin, Q.; Hamilton, A. D. Protein Surface Recognition by Synthetic Receptors: A Route to Novel Sub-micromolar Inhibitors for Chymotrypsin *J. Am. Chem. Soc.* **1999**, *121*, 8-13.
2. Nicholls, P.; Mochan, E. *Biochem. J.* **1971**, *121*, 55-67.
3. Oefner, C., Arey, A.D., Winkler, F.K., Eggimann, B., Hosang, M. Crystal structure of human platelet derived growth factor, *The EMBO Journal*, **1996**, *11*, 3921-3926.

11) Appendices

One paper.

Jain, R.; Hamilton, A. D. "Protein Surface Recognition by Synthetic Receptors Based on a Tetraphenylporphyrin Scaffold", *Organic Letters*, In press.

Protein Surface Recognition by Synthetic Receptors Based on a Tetraphenylporphyrin Scaffold
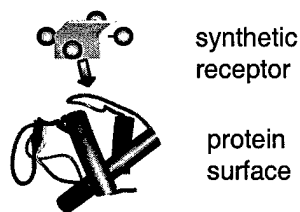
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ABSTRACT

synthetic receptor

protein surface

Receptors based on a tetraphenylporphyrin scaffold bearing different charged and hydrophobic groups have been synthesized. The interactions of these with horse heart cytochrome c were studied by fluorescence spectroscopy. Receptor 4 was identified to be the strongest synthetic receptor (K_d = 20nM) for cytochrome c. The differences in affinity among the receptors reflected a dependence on the number of anionic and hydrophobic groups.

The last two decades have seen enormous progress in the design of synthetic molecules targeted to disrupt protein-ligand interactions.[1] The majority of these medicinally important molecules disrupt interactions occuring inside well-defined cavities on the proteins. In contrast, synthetic molecules that mediate protein function through binding to the solvent exposed *exterior surface* are largely unexplored.[2] This is surprising since such molecules may provide a fundamentally different mechanism for modulating protein function, by sterically preventing protein-ligand and protein-protein interactions. This strategy may not only result in the discovery of novel drug candidates, but may also provide opportunities to systematically understand the role of protein exterior surfaces in molecular recognition.

Recently, we have reported a novel family of synthetic receptors that target protein exteriors.[3] The initial design, involving four peptide loops arrayed around a central calix[4]arene core, was shown to inhibit the approach of small molecule substrates to cytochrome c and chymotrypsin at submicromolar concentrations. However, the calixarene unit suffers several complications, including difficulty of synthesis and tendency to aggregation. We sought to improve this synthetic approach by studying different organic scaffolds onto which recognition sites can be attached. Porphyrins, owing to their large size, rigidity and photophysical properties, have been used in numerous artificial receptors and model systems in bioorganic and bioinorganic chemistry.[4] In this Letter, we report on the design, synthesis and recognition properties of a series of

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(2) (a) Cushman, M.; Kanamathareddy, S.; De Clerq, E.; Schols, D.; Goldman, M.E.; Bowen, J.A. *J. Med. Chem.* **1991,** *34,* 337-342. (b) Regan, J.; McGarry, D.; Bruno, J.; Green D.; Newman, J.; Hsu, C.Y.; Kline, J.; Barton, J.; Travis, J.; Choi, Y.M.; Volz, F.; Pauls, H.; Harrison, R.; Zilberstein, A.; Ben-Sasson, S.A.; Chang, M. *J.Med.Chem.* **1997,** *40,* 3408-3422.

(3) (a) Hamuro, Y.; Calama, M. C.; Park, H. S.; Hamilton, A. D. *Angew. Chem. Int. Ed. Engl.* **1997,** 36, 2680-2683. (b) Park, H.S.; Lin, Q.; Hamilton, A.D. *J. Am. Chem. Soc.* **1999,** 121, 8-13. (c) Lin, Q.; Park, H.S.; Hamuro,Y.; Lee, C.S.; Hamilton, A.D. *Biopolymers* **1998** *,47,* 285-297.

(4) Ogoshi,H.; Mizutani,T. *Curr.Opin.Chem.Biol.* **1999,** *3, 736-739.*

tetraphenylporphyrins that bind, in certain cases with high affinity, to the surface of cytochrome c.

Cytochrome c is a particularly attractive target since it plays key roles in electron transfer and apoptosis, that are mediated by complex formation to other proteins (cytochrome c oxidase, Apaf1, etc.). One critical recognition region involves an array of lysine and arginine residues surrounding the exposed heme edge surface.[5] The tetraphenylporphyrin scaffold well matches the arrangement of hydrophobic and charged domains present in this region (Figure 1). In pioneering work, Fisher recognized this geometrical relationship and showed that tetracarboxyphenyl porphyrin **1** binds to cytochrome c with a K_d of ~5μM.[6]
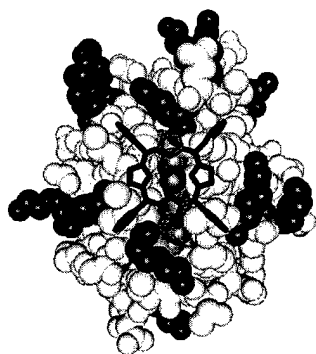

Figure 1. A space filling representation of horse heart cytochrome c based on its X-ray crystal structure.[7] The heme group which directly faces the reader, is drawn in gray spheres. Black spheres represent positively charged lysine and arginine residues. White spheres correspond to all other residues. A tetraphenylporphyrin scaffold (black cylindrical bonds) is drawn in the center.
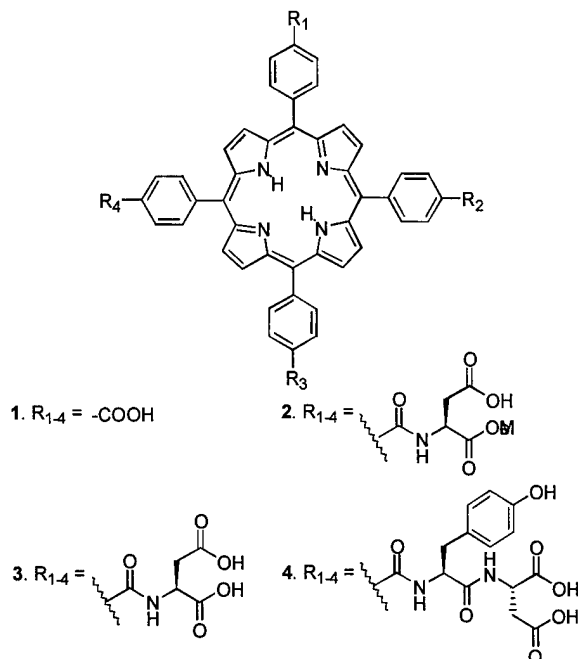
To determine the optimum combination of recognition features in synthetic receptors of this type, we designed a series of tetraphenylporphyrins with various amino acid derivatives attached. The resulting receptors contain a large, flat and semi-rigid molecular surface of approximately 300-400 Å^2 in area. The receptors were prepared by the generation of the tetra-acid chloride ((COCl)$_2$, DMF) starting from m-tetrakis-(4-carboxyphenyl) porphyrin **1**, and subsequent coupling with the corresponding t-butyl protected amino acid or peptide amines. Deprotection of t-butyl groups with trifluoroacetic acid provided receptors **2-4**. Acidic and hydrophobic attachments were chosen to complement the cationic and hydrophobic surface surrounding the heme edge of cytochrome c.

(5) Scott, R.A.; Mauk, A.G. *Cytochrome c a Multidisciplinary Approach;* University Science Books: Sausalito, 1996.

(6) Clark-Ferris, K.K.; Fisher, J. *J.Am.Chem.Soc.* **1985**, *107*, 5007-5008.

(7) Bushnell, G. W.; Louie, G. V.; Brayer, G. D. *J.Mol.Biol.* **1990**, *214*, 585-595.

Compounds **1-4**, and coproporphyrin I, a naturally occuring tetraanionic porphyrin, were initially screened for binding to horse heart ferricytochrome c using fluorescence spectroscopy. Addition of cytochrome c to solutions containing **1-4** resulted in quenching of porphyrin fluorescence emission (ex=420nm, em=650nm) due to the enforced proximity of the Fe(III) heme that results from complex formation between the protein and the receptors.

1. R_{1-4} = -COOH   2. R_{1-4} =

3. R_{1-4} =   4. R_{1-4} =

In contrast, titrations with tetracationic m-tetrakis-(4-trimethylaminophenyl) porphyrin (TTMAPP), showed no quenching even at high concentrations indicating the absence of nonspecific binding. Typical titrations of **3, 4** and TTMAPP with cytochrome c are shown in Figure 2 (see supporting information for others).

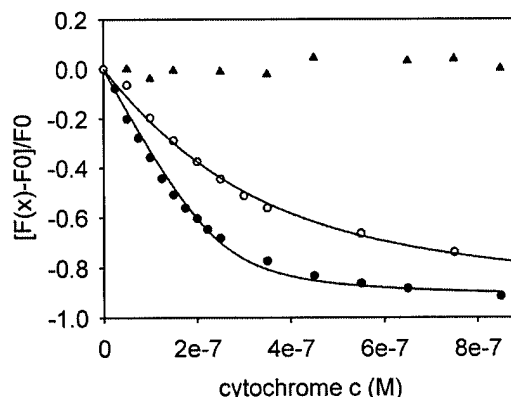**Figure 2.** Fluorescence quenching of **4** (●), **3** (○) and TTMAPP (▲) upon addition of cytochrome c. The curve fit indicates a K_d of 20 ±5nM for **4** and 160 ±20nM for **3**. Titrations were carried out under 250nM initial receptor concentration in 5mM sodium phosphate buffer, pH = 7.4, at 298K.

Dissociation constants were derived by curve fitting to a 1:1 binding equation[8] with stoichiometries being fixed at n=1 for all compounds. Preference for 1:1 binding was confirmed for receptors **3** and **4** by Job's method[9] (see supporting information). K_d for all compounds are summarized in Table 1.

Table 1. Dissociation constants[a] and structural properties of synthetic receptors and other water soluble porphyrins.

compound	K_d (nM)	charge	aryl group
1	950 ±250	- 4	4
2	860 ±90	- 4	4
3	160 ±20	- 8	4
4	20 ±5	- 8	8
coproporphyrin I	7700 ±270	- 4	0
uroporphyrin I	1000[b]	- 8	0

[a] determined at 5mM sodium phosphate, pH 7.4, 298K
[b] from reference 10, at $\mu = 4mM$ sodium phosphate, pH 7.26, 298K

Significant changes in affinity for cytochrome c were observed by altering the relative proportions of acidic and aromatic functionalities in the receptors (Table 1). Receptors **2** and **3** differ only by the substitution of four methyl esters by carboxylic acids, respectively, providing controls for probing the charge requirements for cytochrome c recognition. A ~5 fold increase in affinity was seen, on going from receptor **2** to receptor **3**, indicating a preference for octaanionic receptors over their tetraanionic counterparts. Receptors **1** and **2**, with the same number and type of charged groups, show little difference in their binding affinities. Similar trends were observed when aromatic groups were incorporated into the receptor while keeping the number of charged groups constant. Rodgers had earlier shown that uroporphyrin, containing eight carboxylate groups, binds cytochrome c with µM affinity.[10] However, receptor **3** which has eight anionic groups and 4 phenyl groups binds to cytochrome c approximately six fold stronger than uroporphyrin. In a similar analysis, receptor **2** binds cytochrome c nine fold stronger than tetraanionic coproporphyrin I. These results suggested that an appropriate combination of charged and

hydrophobic groups on the porphyrin periphery would give a molecule with exceptionally high affinity for cytochrome c. To confirm this, we designed receptor **4** to contain eight negatively charged groups and 8 phenyl groups. The titration curve (Figure 1) shows a sharper achievement of saturation with **4** compared to **3**. This corresponds to a K_d of 20 ±5 nM for **4** binding to cytochrome c and represents an eight fold increase in affinity compared to **3** (Table 1).To our knowledge, **4** is one of the strongest synthetic receptors for cytochrome c, under these experimental conditions. The binding experiments between **4** and cytochrome c were carried out in 5mM phosphate buffer at pH 7.4. Remarkably, receptor **4** with a molecular weight of ~1900 Da can bind to cytochrome c 100 fold stronger than its natural protein partners such as cytochrome c peroxidase, which has a K_d of 2.4µM measured at 5mM phosphate buffer, pH=7.[11] The combination of many electrostatic and hydrophobic interactions over a large contact surface is primarily responsible for the formation of high affinity protein-protein complexes in nature.[12] The synthetic receptors reported here behave similarly, attaining large enhancements in affinity through the incorporation of both anionic and aromatic groups. The tetraphenylporphyrin scaffold appears to provide a template in which the peripheral anionic and aromatic groups take up a good geometrical relationship to the cationic and hydrophobic side chains of cytochrome c. Based on these findings, it should be possible to extend this approach to identify new receptors for protein targets that are known to interact with simple porphyrin derivatives.[13]

In summary we have designed and synthesized synthetic receptors that recognize a protein surface with high affinity in aqueous medium. Analogs of receptor **4** with less symmetrical substitution patterns may provide a clearer understanding of the detailed recognition properties of the cytochrome c protein surface. In addition to providing a new strategy for protein surface recognition, these findings may have important medicinal consequences. Cytochrome c has been shown to interact with Apaf1, leading to the activation of programmed cell death or apoptosis.[14] Receptor **4** may serve as a valuable lead in the search for efficient disruptors of Apaf1-cytochrome c interaction.

Supporting Information Available: Experimental procedure and characterization for compounds **2-4**. Fluorescence titration plots for **1-2**, coproporphyrin I and Job's plots for **3** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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